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Enantioselective Polymeric Transporters for Tryptophan, Phenylalanine, and Histidine Prepared Using Molecular Imprinting Techniques

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Developing new methods for the separation of enantiomers is of great current interest because of the importance, challenge, and high cost associated with such separations. This is particularly true in the pharmaceutical industry because of the requirement for the high purity, including enantiopurity, of the final drug products. In this study, polymeric molecular transporters were prepared using molecular imprinting techniques with D-tryptophan, D-phenylalanine, and D-histidine as the templates, respectively. It was found that the transporters thus prepared were able to transport the template amino acids across a hydrophobic chloroform layer in a U-tube at rates that were 1.34- to 3.8-fold higher than the transport of their L-enantiomers. The magnitude of discrimination depends on the conditions of polymerization and the templates used. Molecular ''receptors'' prepared using molecular imprinting techniques could potentially be used for the separation of enantiomers through serial enantioselective transports.

INTRODUCTION

Developing new methods for the separation of enantiomers is of great current interest because of the importance, challenge, and high cost associated with such separations. This is particularly true in the pharmaceutical industry because of the requirement for the high purity, including enantiopurity, of the final drug products (*1*). Recently, Lakshmi and Martin reported a novel method for the separation of D- and L-amino acids using immobilized apoenzymes as enantioselective transporters (*2*). Using this method, a fivefold difference between the transport rates of D- and L- amino acids was achieved. Herein, we report our efforts in developing enantioselective polymeric transporters prepared using molecular imprinting techniques.

Molecular imprinting, first demonstrated by Dickey (*3*), is a technique for making biomimetic polymeric receptors for the target ''print'' molecules (*4–8*). The process consists of (1) mixing the ''print'' compound with monomers with appropriate functional groups, (2) low temperature polymerization, and (3) extracting the ''print'' molecule from the polymer, which leaves cavities inside the polymers that are complementary in terms of size, shape, and functional group orientation to

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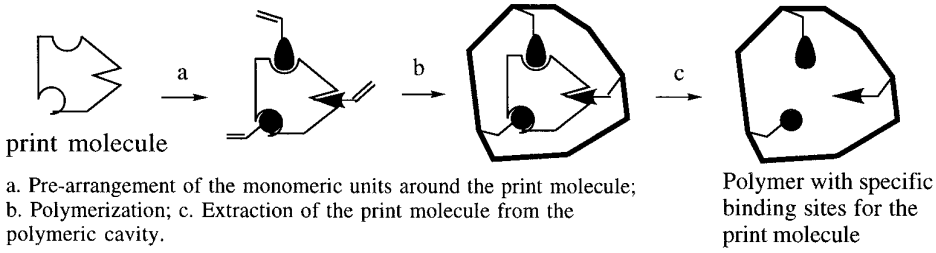

SCHEME 1. A cartoon description of the molecular imprinting process.

those of the "print" molecules (Scheme 1). This technique has been used for the preparation of selective recognition sites for a wide variety of molecules (*6, 8–14*). Recently, a quite sensitive glucose sensor was prepared using this technique which may have the potential for practical applications (*15*). There have also been extensive efforts in developing polymeric receptors that discriminate enantiomers using amino acids or peptides as models. For example, using molecularly imprinted ligand-exchange adsorbents, the chiral discriminations of seven unprotected α-amino acids have been studied. In equilibrium rebinding studies, amino acids with large aromatic side chains, such as phenylalanine, exhibited the highest selectivities ($\alpha = 1.65$) (*7*). Somewhat higher enantioselectivities have been achieved if amide functional groups are used for the imprint polymer preparation (*16*). Using HPLC capacity factors as a measurement of the selective recognition, Mosbach and coworkers have developed amide imprint polymers that have enantioselectivity factors as high as 4 for protected tryptophans (*14, 17*). Using peptides as templates, Mosbach and coworkers also developed polymeric receptors that recognized the LL form of a dipeptide over the DD form with a K_d (dissociation constant) ratio of 1.64/2.00 (mM) (*18*). Using trimethylolpropane trimethacrylate as the cross linking reagent, the enantioselectivities of protected dipeptides were increased to a K_d ratio of 2.5/4.8 (mM) (*19*). Most of these studies were focused on the development of chromatographic materials for separation purposes (*4, 5, 20*).

Two major problems are preventing imprinted polymers being used as chromatographic stationary phases in practice (*4*). First, the slow binding of the template to the polymeric binding sites causes peak broadening, tailing, and consequently decreased separation efficiency (*4, 5, 21*). During a chromatographic process, the separation heavily relies on the dynamic equilibrium constants and a fast exchange is essential for the efficient resolution of two compounds based on their inherent differences in binding affinities to the stationary phase. A slow exchange undoubtedly would not allow for the chromatographic process to take advantage of the specific recognition of the compounds by the polymeric binding sites generated through molecular imprinting. Second, imprinted polymers typically have very heterogeneous binding sites and accessibilities and low functional capacities, which results in separations being performed in the nonlinear part of the adsorption isotherm (*4, 22*). New polymerization methods could be developed in the future to

address some of these problems (*4*). However, there are also other nonchromatographic approaches being tested in examining the practicality of using imprinted polymers for separations. For example, selectively permeable membranes have been prepared using the molecular imprinting method and tested for their applications in separations (*23, 24*). Using selectively permeable membranes does avoid the problems caused by slow binding of the target molecules to the binding sites. However, smaller molecules could still nonspecifically permeate such membranes.

Conceivably, molecular imprinting techniques could also be used to prepare polymeric transporters, which can be used for separations. In such a design, the slow binding of such imprinted polymers would only affect the overall transport rate, but not the transport selectivity, because only the bound molecules could cross the otherwise nonpermeable layer. Furthermore, the heterogeneity of the polymeric receptor sites and particle sizes should not be a problem either in a transport system because it should not affect the overall transport selectivity as it affects the peak shape and tailing and, consequently, resolution in a chromatographic system. Therefore, we undertook this study to examine the possibility of preparing polymeric transporters using the molecular imprinting techniques. We have prepared polymeric transporters for D-tryptophan, D-phenylalanine, and D-histidine that can selectively transport one enantiomer over the other with about 1.34- to 3.8-fold differences depending on the polymerization conditions and the template used.

RESULTS AND DISCUSSION

Synthesis of polymers. We decided to use 3,3-dimethylacrylate (DMA)-based polymers using ethyleneglycol dimethacrylate (EGDM) as the cross linking agent for the preparation of highly cross-linked polymers. This is because similar polymers have been widely used in molecular imprinting studies (*4, 5*). AIBN [2,2′-azobis-(2-methylpropionitrile)] was used as the initiating reagent for the free radical polymerization. D-Tryptophan, D-phenylalanine, and D-histidine were used as the templates, the ''print'' molecules. There are two polymerization methods that have been commonly used in preparing imprinted polymers of similar types (*4*). One is a thermal method, where polymerization was initiated by thermally initiating the generation of free radicals from AIBN. The second method is a photo method, where UV is used for the generation of free radicals from AIBN. One advantage of the photo method is that the polymerization can be carried out at a lower temperature, which presumably helps to maximize the intermolecular interactions for the optimal orientation of all the necessary functional groups (*22, 25*). In our studies, we employed both the thermal and the photo methods aimed at examining the effect of polymerization temperature on the selectivity of the molecular transporters prepared.

We first prepared the D-tryptophan-imprinted polymers using three different polymerization conditions. In the photo method, we used a slightly modified procedure. Instead of a commonly used high-powered UV lamp (about 500 watts), we selected a low-powered (4 watts) pen-like UV lamp that can be inserted into the reaction vessel. This method offers the following advantages. First, because the

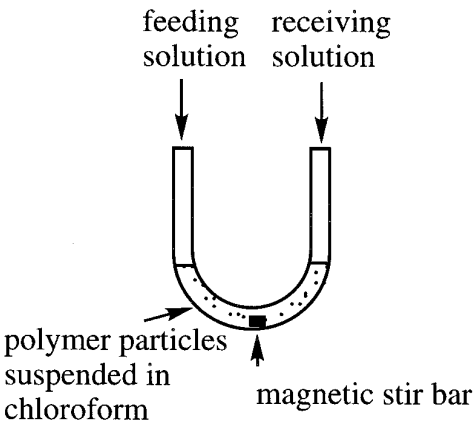

FIG. 1. Transport study apparatus set-up.

light only generates a small amount of heat, the temperature of the polymerization reaction can be easily controlled using a regular ice-bath or the polymerization can be carried out in a cold-room without other cooling method. Second, the reaction set-up is easy and does not require special photochemical reactors or special quartz glassware. Thus, with this method, polymerization was carried out at 0–4°C for about 24 h with a DMA to EGDM ratio of 1 : 10 using AIBN as the initiator. For the thermal method, the polymerization was carried out at 45–50°C for about 48 h and then at 65°C for about 2 h. We also prepared polymers using a combined method of UV irradiation followed by thermal polymerization. In all cases, the polymers prepared were dried in a vacuum oven (at 45°C) for at least 4 h and then ground to fine particles. The print molecules were then extracted with a mixture of methanol and acetic acid with a ratio of 9 : 1 until no amino acid absorbance could be detected in the washing solution. The percentage of the recovered tryptophan through extraction of the imprinted polymers was also determined by measuring the UV absorption of solution. In all the experiments, over 90% of the tryptophan was recovered. Then the particles were sieved using a 100-mesh sieve and particles smaller than 100 mesh were used.

Because the photopolymerization method gave the best results in transport studies (see next section) with the D-tryptophan imprinted polymers, subsequent preparation of D-phenylalanine and D-histidine imprinted polymers employed only the photo-induced polymerization method.

Transport studies. The ability for these polymers to enantioselectively transport the corresponding amino acid across an organic phase was examined by using a U-shaped tube as it is often used in similar transport studies (Fig. 1) (*26*). In such a design, 8 ml of chloroform was used to suspend the polymer particles (10 mg/ml). On the feeding side was 20 mM of the amino acid in a 0.03 M citric acid aqueous solution. The pH of the feeding side aqueous phase was 2.7. This was done because acid was used to dissolve the amino acid before polymerization and, therefore, the

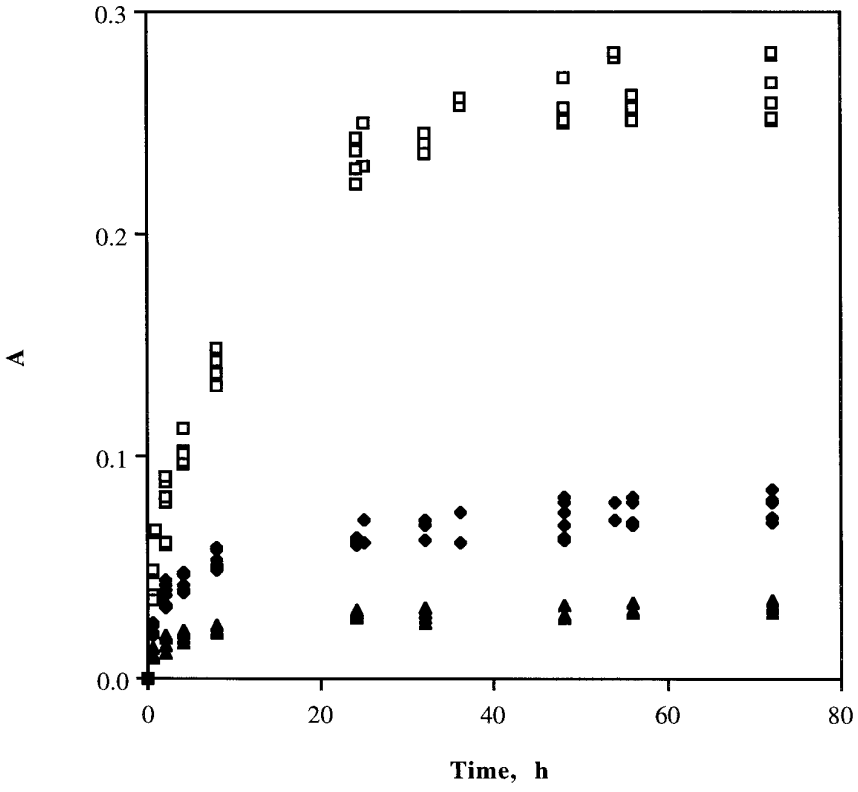

FIG. 2. The UV absorbance (220 nm) of the receiving side aqueous solution at different time. The polymers were made by using the UV-induced polymerization method with D-tryptophan as the template. (□) D-Trp; (◆) L-Trp; (▲) control polymer as the transporter for the transportation of D-tryptophan.

polymers prepared would best recognize the free acid form instead of the form which has the carboxylate anion. Constant suspension of the imprinted polymer particles in $CHCl_3$ was achieved by magnetic stirring at the bottom of the U-tube. The UV absorbance of the aqueous solution on the receiving side was measured at different intervals to monitor the transport of the amino acid. The transport experiments were carried out for a period of at least 72 h. The concentration of the amino acid in the receiving solution was calculated based on the extinction coefficient of each amino acid under that condition. Figures 2–4 show the time profiles of the transports of D- and L-tryptophan using polymers imprinted with D-tryptophan using the three polymerization methods mentioned above. From these figures it can be clearly seen that all the polymers showed selective transport of the print molecule (D-tryptophan) over its enantiomer (L-tryptophan). Control polymers prepared in the absence of the template, D-tryptophan, did not transport D-tryptophan at an appreciable rate (Figs. 2–4). This further indicates that it is the special recognition site built through the molecular imprinting process that is

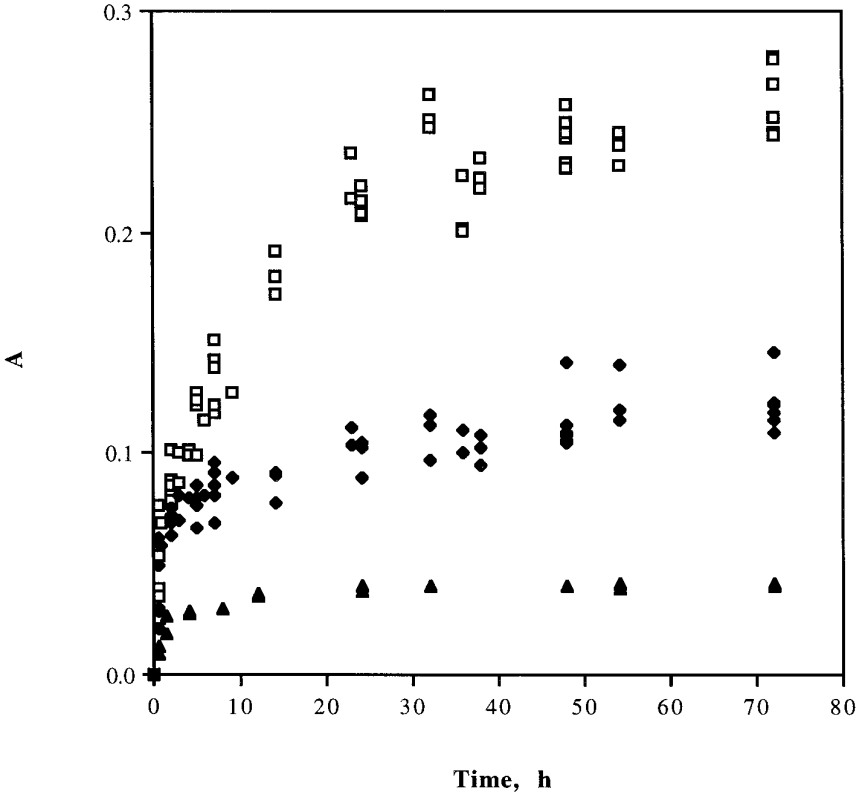

FIG. 3. The UV absorbance (220 nm) of the receiving side aqueous solution at different time. The polymers were made by using the thermal polymerization method with D-tryptophan as the template. (□) D-Trp; (◆) L-Trp; (▲) control polymer as the transporter for the transportation of D-tryptophan.

responsible for the selective transport. To quantify the enantioselectivity, the final concentration of tryptophan at the 72 h point (Table 1) is used to compare the enantioselectivity in transport. The transport rates at the relatively linear region (the first 10 h) were also calculated (Table 2). All polymers exhibited selective transport of the print molecule over its enantiomer. For example, polymers prepared using the photo method at 0°C with D-tryptophan as the template were able to transport D-tryptophan at an average rate of $(5.29 \pm 0.48) \times 10^{-8}$ mol/h.cm^2, which is about 3.04 ± 0.45-fold higher than that of L-tryptophan [$(1.74 \pm 0.46) \times 10^{-8}$ mol/h.cm^2]. If the final concentration at 72 h time point is used as a measurement of the enantioselectivity in transport, the results are similar. When the polymer prepared using the photo method at 0°C with D-tryptophan as the template was used as the transporters, the concentration of D-tryptophan on the receiving side of the U-tube reached about 0.236 ± 0.030 mM at the 72 h time point, which is about 3.81 ± 0.33 times higher than that of the L-tryptophan (0.062 ± 0.024 mM) (Table 1). As expected (*22, 25*), the enantioselectivity of the polymeric

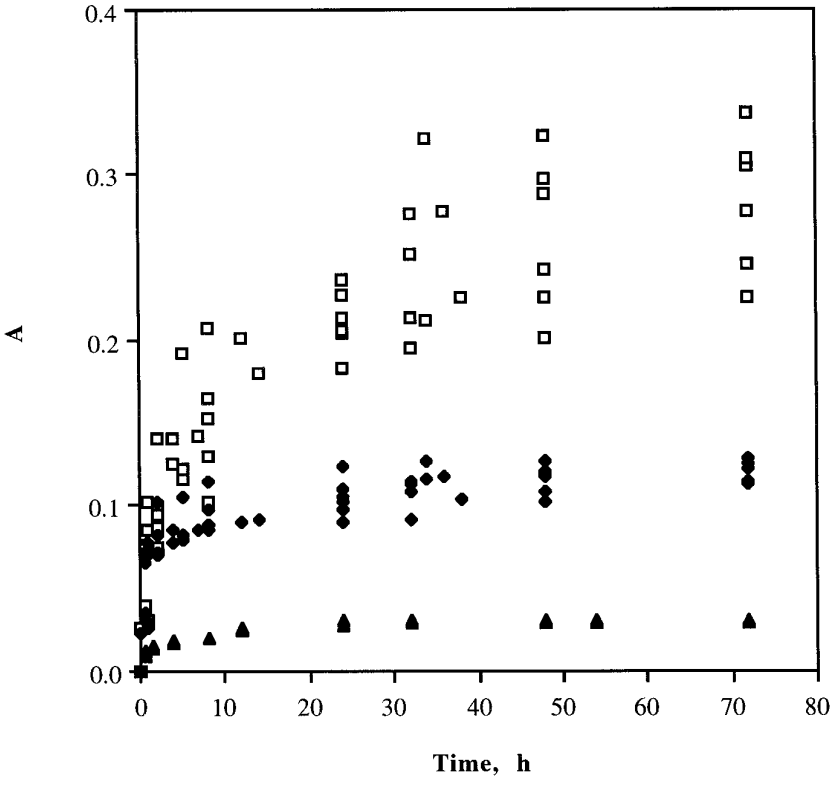

FIG. 4. The UV absorbance (220 nm) of the receiving side aqueous solution at different time. The polymers were made by using a combined photothermal polymerization method with D-tryptophan as the template. (□) D-Trp; (◆) L-Trp; (▲) control polymer as the transporter for the transportation of D-tryptophan.

transporters prepared at a higher temperature was lower than that prepared at 0°C and the results were similar whether the initial transport rates (Table 2) or the concentrations at the 72 h time point (Table 1) were used for the comparison. For example, the enantioselectivity (D/L) of the polymeric transporters prepared at

TABLE 1
Concentration of Tryptophan in the Receiving Side of the U-Tube at the 72-h Time Point

Polymer preparation	D-Trp (mM)	L-Trp (mM)	Ratio
Photo method	0.236 ± 0.030	0.062 ± 0.024	3.81 ± 0.33
Thermal method	0.214 ± 0.016	0.102 ± 0.015	2.10 ± 0.31
A combination of the photo and thermal method	0.190 ± 0.021	0.099 ± 0.004	1.92 ± 0.21